## (19) World Intellectual Property **Organization**

International Bureau





(43) International Publication Date 13 January 2005 (13.01.2005)

PCT

(10) International Publication Number WO 2005/003354 A3

- (51) International Patent Classification<sup>7</sup>: C12N 15/63, 15/31, C12Q 1/00, C12R 1/23, 1/46, A23C 19/00, C12N 15/74
- (21) International Application Number:

PCT/NL2004/000474

(22) International Filing Date:

2 July 2004 (02.07.2004)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

03077074.7

2 July 2003 (02.07.2003)

- (71) Applicant (for all designated States except US): FRIES-LAND BRANDS B.V. [NL/NL]; Blankenstein 142, NL-7943 PE Meppel (NL).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): DEN HENGST. Christiaan, Daniël [NL/NL]; Oliemuldersweg 172, NL-9713 VK Groningen (NL). GAJIC, Olivera [YU/NL]; Esdoornlaan 7, NL-6721 CG Bennekom (NL). KUIPERS, Oscar, Paul [NL/NL]; Lepelaar 95, NL-9728 XG Groningen (NL). KOK, Jan [NL/NL]; Van Hamelstraat 34, NL-9714 HL Groningen (NL). SIKKEMA, Jan [NL/NL]; Hoofdweg 46, NL-9483 PC Zeegse (NL). GEURTS. Johannes, Marie, Wilhelmus [NL/NL]; Antwerpenstraat 18, NL-7559 NK Hengelo (NL). NAUTA, Arjen [NL/NL]; Esdoornlaan 7, NL-6721 CG Bennekom (NL).

- (74) Agent: WINCKELS, J., H., F.; Vereenigde, Johan de Wittlaan 7, NL-2517 JR Den Haag (NL).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

## Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- (88) Date of publication of the international search report: 7 April 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS AND MEANS FOR REGULATING GENE EXPRESSION

(57) Abstract: The invention relates to the field of biochemistry, molecular biology and food production. More in particular, the invention relates to methods and means for regulating gene expression. Even more in particular, the invention relates to CodY target sequences. Herein we show that CodY represses its target genes by binding to specific DNA sequences upstream of the respective genes. A conserved target site was identified by analyzing upstream sequences of derepressed genes in a delta codY L. lactis MG1363 derivative, as identified in a DNA micro-array study. The present application furthermore discloses CodY target sequences from other gram-positive bacteria, like B. subtilis and Streptococcus. Hence, the invention provides CodY target sequences that may be used in different applications to repress or derepress gene expression.

